Poster

368. Imaging Advances: New Histology, Reagents, and Approaches

Location: Halls A-C

Time: Monday, November 17, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 368.06/UU84

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: FWF Grant P 23102-N22

Title: Optics for the imaging of cleared samples

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Abstract: In recent years chemically cleared biological samples like cleared mouse brains, mouse embryos, lymph nodes or tumors have become increasingly popular. These preparations can be imaged in 3D in toto with cellular resolution with suitable microscopes like the ultramicroscope we developed (1). This allows one to answer many biological questions which necessitate the 3D reconstruction of the sample. However these cleared preparations have all non standard optical refractive indices and must be imaged in media with the same refractive index. These range from 1.38 to 1.56 and specialized objectives must be used for these applications. Up to now they are only available for a limited range of refractive indices, have small fields of view and are extremely costly. We have thus taken another approach and developed specialized correction devices which allow the use of existing air objectives both for the 3DISC0 (2,3) (n=1.56) and the CLARITY (n=1.45) clearing. Objective magnifications ranged from 2X to 20X and N.A.s from 0.14 to 0.6. with a working distance of at least 10 mm to image through whole mouse brains. As objectives can be easily changed in the ultramicroscope, it is possilble to get a quick overview of the whole brain in 3 D and then image regions of interest with higher resolution. We were thus able to image hippocampal neurons with their dendrites inside whole mouse brains allowing 3D reconstructions. Also whole *drosophilae* could be easily imaged. In addition we have been able to bring down the thickness of the light sheet in the ultramicroscope to the 1 μm range with specialized aspheric optics. This is pivotal for isotropic imaging as in other existing light sheet microscopes generally laterally resolution is much better than axial resolution. For biological objects lager than mouse brains this will be of utmost importance. 1. Dodt HU, Leischner U, Schierloh A, Jährling N, Mauch CP, Deininger K, Deussing JM, Eder M, Zieglgänsberger W, Becker K (2007) Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain, Nat. Meth 4: 331-336 2. Becker K, Jährling N,

Saghafi S, Weiler R, Dodt HU (2012) Chemical clearing and dehydration of GFP expressing mouse brains, PLoS One 7: e33916 3. Ertürk A, Becker K, Jährling N, Mauch CP, Hojer CD, Egen JG, Hellal F, Bradke F, Sheng M, Dodt HU (2012) Three-dimensional imaging of solvent-cleared organs using 3DISCO. Nat Protoc 7: 1993-95

Disclosures: H. Dodt: None. **K. Becker:** None. **C. Hahn:** None. **N. Jährling:** None. **S. Saghafi:** None.

Poster

368. Imaging Advances: New Histology, Reagents, and Approaches

Location: Halls A-C

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Clearing of the mouse temporal bone using a modified SeeDB protocol

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Abstract: Background: Histological and anatomical studies of the mouse inner ear are technically difficult due to its small size and its location embedded in the temporal bone. A recently published protocol, SeeDB (Ke et al., *Nature Neuroscience* 16, 1154-1161(2013)) (http://www.nature.com/neuro/journal/v16/n8/full/nn.3447.html), allows the clearing and the visualization of a whole brain in mice in a 3D fashion. Our goal was to modify the SeeDB protocol to achieve 3D visualization of inner ear organs encased in the temporal bone. We sought to establish the optimal protocol for clearing bone and cartilage in the mouse temporal bone to be suitable for immunofluorescent labeling. Method: Temporal bones were dissected from mice at either postnatal day 1 - 2 (P1-2) or at P30. The SeeDB protocol was modified for the mouse temporal bone. Briefly, the adult temporal bones were decalcified before starting the process. The cartilaginous temporal bone of younger mice was processed directly without decalcification. After fixation in 4% paraformaldehyde in PBS, the samples were labeled with hair cell-specific markers. Then the samples were incubated in a series of fructose solutions with increasing concentrations. The cleared samples were labeled with fluorescent markers including Neurotrace Fluorescent Nissl Stain, Phalloidin, and Hoechst. Samples were mounted and viewed using fluorescent microscopy. Results: Successful clearing of cartilaginous temporal bone and partial clearing of decalcified temporal bone was achieved. Conclusion: Our modified version of the SeeDB protocol was low-cost using readily available materials. Using this protocol, we were